UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

Date: October 5, 2016

Subject: **Thiabendazole:** HED review of a developmental thyroid study in rats.

PC Code: 060101
Decision No.: 499972
Petition No.: NA
Risk Assessment Type: NA
TXR No.: 0057512
MRID No.: 49928301, 49928302

DP Barcode: D434965
Registration No.: 100-963
Regulatory Action: NA
Case No.: NA
CAS No.: 148-79-8
40 CFR: NA

FROM: Abdallah Khasawinah, Ph.D., Toxicologist
Risk Assessment Branch IV
Health Effects Division (7509P)

THROUGH: Elissa Reaves, Ph.D., Chief
Risk Assessment Branch IV
Health Effects Division (7509P)

10/5/16

TO: Tony Kish, Risk Manger
Fungicide Branch (FB)
Registration Division RD/OPP (7505P)

I. ACTION: Review developmental thyroid study (MRID 49928301, 49928302).

II. BACKGROUND: A comparative thyroid study was required to fulfil a data registration gap. The registrant conducted this study using one single oral dose based on the existing database showing 10 mg/kg/day as the lowest NOAEL and based on a protocol outline that was reviewed by the Agency (TXR 0057235). The purpose of this study was to determine if there was selective sensitivity of the developing rat to thiabendazole-induced changes in thyroid homeostasis at the current POD of 10 mg/kg/day. The Agency also required a positive control group to produce at least a 30% response but not greater than 50% in the thyroid hormones.

The study was conducted using three groups: test chemical (thiabendazole at 10 mg/kg/day), vehicle control and a positive control group 5-propyl-2-thiouracil (5-PTU) at a dose level of 1.2 mg/kg/day (MRID 49928301).

The study report was received by the Agency, reviewed and found to be unacceptable based on:

- The lack of any positive findings in the positive control group.
- Pups were not dosed directly. It was assumed pups were exposed in utero and postnatally. No data was presented to demonstrate this.
- Limited fetal data was presented. Only hormonal data was presented.

The registrant submitted a supplement report (MRID 49928302) attributing the lack of a positive response to, "Either the dose was not adequate for the rat's genetics/stock/strain, or the rats did not receive the proper dose due an undetected problem with the PTU chemical and/or formulation". The supplementary report also provided a summary table of the use of 3.8 mg/kg/day of PTU (did not specify 5-PTU or 6-PTU) where substantial response was reported.

It is noted that the study employed 5-PTU as a positive control agent. The use of this agent was based on a published paper by Axelstad et al. 2008 as cited in the study report. Examination of the reference paper indicated the use of 6-propyl-2-thiouracil (6-PTU) where this agent produced a positive response on the thyroid gland and the thyroid hormones. It is noted that 6-PTU is the standard agent in many published studies and several studies that have been received and reviewed by the agency.

III. CONCLUSIONS: This developmental thyroid toxicity study/Comparative Thyroid Assessment study (CTA) in the rat was peer reviewed by HED toxicologists (ToxSAC) on September 15, 2016 and concluded to be **Unacceptable**. The positive control agent employed in the study showed no response, which makes the test chemical group results not reliable. Additionally, there was no proof that the test system was working. ToxSAC noted that:

- A new study should be conducted using 6-PTU, or an adequate response from 5-PTU must be demonstrated in the positive control group.
- The structural differences between 5- and 6-PTU may lead to profoundly different effects.
- There are no references for the use of 5-PTU as a positive control.
- The registrant cited a reference on the 6-PTU and not for 5-PTU.
- The registrant did not provide 5-PTU positive control data from the lab. The supplementary information included only a summary table of a PTU agent (the PTU isomer was not specified) at 3x of the dose used in the study.

EPA Reviewer: Abdallah Khasawinah, PhD **Signature:**
Risk Assessment Branch IV, Health Effects Division (7509P) **Date:** 10-05-2016
EPA Secondary Reviewer: Elizabeth Mendez, PhD. **Signature:**
Health Effects Division (7509P) **Date:** 10/6/16 for

Template version 09/11

TXR#: 0057512

DATA EVALUATION RECORD

STUDY TYPE: Developmental Thyroid Study – Rats; Special Study

PC CODE: 060101 **DP BARCODE:** D434965

TEST MATERIAL (PURITY): Thiabendazole (99.5% a.i.)

SYNONYMS: .

CITATION: Coder, Prägati Sawhney. (2016) Thiabendazole – An Oral (Gavage) Comparative Thyroid Assay in Pregnant, Postnatal and Fetal Sprague Dawley Rats. Charles River Laboratories Ashland, LLC, Ohio. Laboratory report number WIL-639235; July 25, 2016. MRID 49928301. Unpublished.

Minnema, D. (2016) Thiabendazole – Validity of the Developmental Thyroid Toxicity Study Assessment. Syngenta Crop Protection, LLC, Greensboro, NC. Report Number: TK0249389. July 25, 2016. MRID 49928302. Unpublished.

SPONSOR: Syngenta Ltd, Jealott's Hill International Research Centre. Bracknell, Berkshire, RG42 6EY UK.

EXECUTIVE SUMMARY:

In a developmental thyroid toxicity study (MRID 49928301), thiabendazole technical (99.5% a.i., lot# 537553) was administered to 40 pregnant female Sprague Dawley [Crl:CD(SD)] rats/dose by oral gavage in 0.5% (w/v) methylcellulose in deionized water (10 mL/kg body weight) at 0 or 10 mg/kg bw/day. 5-Propyl-2-thiouracile (PTU) was administered in corn oil at a dose of 1.2 mg/kg/day in a dose volume of 2 mL/kg to a similar number of pregnant rats (positive control group). Doses were adjusted based on the most recently recorded body weight and administered at approximately the same time on each day. Females were dosed once daily from gestation day (GD) 6 through GD 19 (Subset I; 20 females/group) or from GD 6 through lactation day (LD) 20 (Subset II; 20 females/group). Females that failed to deliver were dosed through post-mating day 24. Females were approximately 14 weeks of age at the beginning of test substance administration.

All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights, and food consumption were recorded at appropriate intervals. Subset I females were sacrificed on GD 20. Subset II females were allowed to deliver and to rear their offspring to LD 21. Clinical observations, body weights, and sexes were recorded for pups at appropriate intervals. On post-natal day 4 (PND 4), pups were culled to 8 pups/litter (4/sex). These pups were

reared to PND 21 and were not directly dosed with the test material or the positive control agent 5-PTU. The remaining pups were euthanized and used for specimen collection.

Blood samples were collected for thyroid hormone analysis (T3, T4, and TSH) from Subset I females on GD 20 or LD 21 (Subset II), from fetuses on GD 20, and from Subset II litters (culled pups on PND 4 and selected pups on PND 21; 2 pups/sex/litter). For all Subset I and Subset II females, the liver and thyroid glands were weighed and retained. All fetal carcasses were retained. For all pups (culled pups on PND 4 and selected pups on PND 21), the liver was weighed and the liver and thyroid were retained; the remaining carcasses were also retained. The thyroids and parathyroids from Subset I and II females, fetuses (1 fetus/litter), culled PND 4 pups (1 pup/litter), and selected PND 21 pups (1 pup/sex/litter) were examined microscopically.

There were no adverse effects on body weight, body weight gain, or food consumption in adult females treated with thiabendazole by oral gavage at 10 mg/kg/day during the gestation and lactation phase. No mortality or adverse clinical toxicity was observed either in the thiabendazole treated animals or the PTU positive control animals.

There were no effects of thiabendazole, or the positive control substance on mean maternal gestation length, the process of parturition or the growth, survival, and clinical condition of pups during the postnatal period. In addition, there were no noted treatment-related macroscopic findings in adult animals.

Thiabendazole at 10 mg/kg/day or 5-PTU at 1.2 mg/kg/day did not impact the serum thyroid hormone levels. The serum thyroid hormone levels of dams (gestation and lactation) in the test material treated groups and positive control were comparable with the concurrent vehicle control group. There were no test substance-related alterations in T3, T4, or thyroid stimulating hormone (TSH) levels, thyroid gland or liver weights, or microscopic changes in the thyroid and parathyroid glands of maternal animals (GD20/LD21), fetuses, or pups on PND 4 /PND 21.

This developmental thyroid toxicity study in the rat (pregnant, fetus, offspring) is classified **Unacceptable/Non-Guideline**. The positive control agent 5-PTU used in the study did not produce the expected effects on the thyroid/parathyroid and the T3, T4, TSH hormones. Therefore, in the absence of positive findings in the positive control group animals, the results of the test material are not reliable. Additionally, there was no proof that the test system was working.

Additional information submitted by the registrant in MRID 49928302 did not provide evidence to the lack of response of the positive control agent in the developmental thyroid study. It stated that “the expected response to PTU treatment was not observed. Either the dose was not adequate for the rat’s genetics/stock/strain, or the rats did not receive the proper dose due an undetected problem with the PTU chemical and/or formulation”.

Note. The study was peer reviewed by HED toxicologists (HED ToxSAC) and they concurred with the reviewers conclusions. HED peer reviewers commented:

- A new study should be conducted using 6-PTU, or an adequate response from 5-PTU must be demonstrated in the positive control group.
- The structural differences between 5- and 6-PTU may lead to profoundly different effects.
- There are no references for the use of 5-PTU as a positive control.

- The registrant cited a reference on the 6-PTU and not for 5-PTU.
- The registrant did not provide 5-PTU positive control data from the lab. The supplementary information included only a summary table of a PTU agent (the PTU isomer was not specified) at 3x of the dose used in the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Thiabendazole technical.

Description: Off-white powder

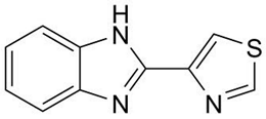
Lot/Batch number#: 537553

Purity: 90.5% a.i., Expiration date (retest date):31- October-2016

Compound stability: Stored at room temperature

CAS #: 148-79-8

Structure:



2. Positive control: 5-propyl-2-thiouracil (PTU)

Description: White powder

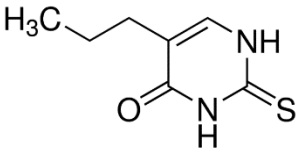
Lot/Batch number#: 034K0980

Purity: 99%, Expiration date (retest date): Jan-2019

Compound stability: Stored at room temperature

CAS #: 2954-52-1

Structure:



3. Vehicle: 0.5% (w/v) methylcellulose in deionized water and corn oil were selected as vehicle for the test material and positive control, respectively.

3. Test animals:		
	Species:	Rats, virgin females
	Strain:	Sprague Dawley [Crl:CD(SD)]
	Age/weight at study initiation:	13 weeks / 223-287 g
	Source:	Charles River Laboratories, Inc., Raleigh, NC
	Housing:	All animals were housed individually in clean, solid-bottom cages with heat-treated aspen bedding material (Northeastern Products Corp.). During the mating period, the animals were housed in groups of two animals/cage (one male and one female) in the home cage of the male.
	Diet:	Certified PMI Nutrition International, LLC Certified Rodent LabDiet® 5002, *ad libitum*
	Water:	Reverse osmosis-purified (on-site) drinking water, *ad libitum*
	Environmental conditions:	**Temperature:** 20.9-22.0°C **Humidity:** 38.4-51.6% **Air changes:** 10/hr **Photoperiod:** 12 hrs dark/12 hrs light
	Acclimation period:	14 days.

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates:** Start: October 6, 2015; End: December 5, 2015.

2. **Mating:** After the acclimatization period, female rats were cohabitated with untreated, sexually mature male rats (1:1) until the requisite numbers of mated females were obtained. Detection of mating was confirmed by evidence of a copulatory plug in the vagina and by a vaginal lavage for sperm. After confirmation of mating, the female animals were returned to their respective cage, and the day was designated as day 0 of gestation (GD-0). Care was taken to avoid sibling mating in the parent (F0) generation animals.

3. **Study design and animal assignment:** Mated female animals were assigned to dose groups by stratified randomization on the basis of GD-0 body weights in a block design.

   There were two subsets of the study as shown in Table1: the gestation dams, lactation dams in addition to PND4 and PND 21 pups.

TABLE 1. Study design [a]

	Dose mg/kg/day		
	Vehicle Control	PTU	Thiabendazole
	0	1.2	10.0
Subset 1: Gestation dams GD 6-19	20	20	20
Subset 2: Lactation dams GD 6-LD 21	20	20	20
PND 4 pups	All culled pups	All culled pups	All culled pups
PND 21 pups	2 pups/sex/litter	2 pups/sex/litter	2 pups/sex/litter

[a] Data obtained from pages 22-26 of the study report (MRID 49928301).

   Individual animals were uniquely identified using a programmable microchip (BMDS system) which was implanted subcutaneously in the dorsoscapular region during the acclimation period.

4. **Dose selection rationale:** The dose levels were selected in consultation with the Sponsor after examining the existing toxicity database for thiabendazole and following discussions between the Sponsor and the United States EPA (United States EPA, 2015, Memorandum, TXR number: 0057028). The purpose of this study was to determine if there was a selective sensitivity of the developing rat to thiabendazole-induced changes in thyroid homeostasis at the current POD of 10 mg/kg/day.

   A positive control group was included to produce at least a 30% response but not greater than 50% - as required by the US EPA during its review of the study protocol (TXR 0057235). To address this requirement, the oral (gavage) administration of 5-Propyl-2-thiouracil (5-PTU) at a dose level of 1.2 mg/kg/day, in the dosing vehicle corn oil, at the constant dose volume of 2 ml/kg was used. Axelstad *et al.* 2008, employing a comparable dosing regimen, demonstrated a decrease in T4 levels of approximately 50% in PND 16 pups, relative to controls. At this PTU dose level, a 47% increase in thyroid weight, along with histopathological changes (15/33 animals) were noted in the PND 16 pups. This dosing regimen was used in the current study as the dose route and frequency mimics that of the test substance. However, the EPA reviewer notes that Axelstad *et al* used **6-Propyl-2-thiouracil (6-PTU)** and not **5-Propyl-2-thiouracil (5-PTU).**

The offspring were not directly exposed to the test substance or positive control substance at any time during the study; the offspring were *potentially* exposed to the test substance *in utero* and while nursing. However, the registrant did not demonstrate transfer of either compound across the placenta or through the milk. The guidance study design provided by the agency states that "Unless specific data are provided that demonstrate sufficient exposure of the pups during lactation, a separate test group should be included that directly doses pups to ensure that there is sufficient exposure for a postnatal evaluation."

5. **Dosage preparation:** The thiabendazole vehicle suspension (0.5% [w/v] methylcellulose in deionized water) was prepared weekly for administration to the vehicle control group and for preparation of the thiabendazole formulations; aliquots were prepared for daily dispensation to the vehicle control group and stored refrigerated. The 5-PTU vehicle (corn oil) was dispensed as necessary for preparation of the 5-PTU formulations.

   The test substance and positive control substance formulations were prepared weekly, divided into aliquots for daily dispensation, and stored refrigerated (test substance formulations) or refrigerated, protected from light (positive control substance formulations). Formulations were stirred continuously throughout the preparation, sampling, and/or dose administration procedures.

   Samples for concentration and/or homogeneity assessment were collected from the top, middle, and bottom strata of the first test substance dosing formulations and from the middle stratum of the first vehicle control group formulation. Samples for concentration analysis were collected from the middle of the last test substance and vehicle control formulations. One set of samples from each collection was subjected to the appropriate analyses. All remaining samples were stored refrigerated (approximately 2°C to 8°C) as back-up.

   Test substance resuspension homogeneity and stability following 10 days of refrigerated storage (2°C to 8°C) was established for a test substance concentration in the vehicle of 1 mg/mL in a previous study (WIL-639236 – was not available for review). Therefore, resuspension homogeneity and stability were not assessed in this study.

   Results:
   Homogeneity and concentration analysis: The mean percent recovery obtained for test material dose formulations was within the WIL Research Lab SOP range for suspensions (85% to 115%). Results showed mean% of target to be 100% with a relative standard deviation (RSD) of 0.71%.

   The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

6. **Dosage administration:** All doses (vehicle, 5-PTU or test material) were administered once daily by oral gavage from GD 6 through GD 19 (Subset I) or from GD 6 through LD 20 (Subset II). Dosing volume was 10 mL/kg for test substance and vehicle control formulations and 2 mL/kg for positive control substance formulations. Subset I females received a total of 14 doses. Subset II females received a total of 36 to 37 doses. Females that failed to deliver

were dosed through postmating day 24 for a total of 19 doses. Doses were adjusted based on the most recently recorded body weight and administered at approximately the same time on each day.

C. OBSERVATIONS:

1. **In life observations (parental animals):** All dams were observed twice daily for mortality, morbidity and daily for clinical signs 1 hour following dosing. Body weights, body weight gains, and food consumption were recorded as detailed in Table 2 for each Subset animals.

2. **In life observations (pups):** Each litter was examined daily for survival, and all deaths were recorded. All pups were individually identified by application of tattoo markings on the digits following completion of parturition. A daily record of litter size was maintained. Offspring found dead were discarded without examination. Clinical observations, body weights and sex determinations were determined on days described in Table 2. Pups were culled to 4/sex litter on PND 4. Standardization of litter size was not performed on litters with fewer than 8 pups. All selections were performed by computerized randomization. Culled pups were weighed and used for PND 4 specimen collection.

TABLE 2. In-life observations and Sacrifice [a]

Observation	Study Phase		
	Gestation Dams [b]	Lactation Dams	Pups [c]
Body weight	GD 0, 6, 9, 12, 15, 18, 20	GD 0, 6, 9, 12, 15, 18, 20 LD 1, 4, 7, 10, 14, 17, 21	PND 1, 4, 7, 10, 14, 17, 21.
Food consumption	GD 0, 6, 9, 12, 15, 18, 20	GD 0, 6, 9, 12, 15, 18, 20 LD 1, 4, 7, 10, 14, 17, 21	-
Sex determination	-	-	PND 0, 4, 14, 21
Sacrifice time -Dams	GD20	LD21	-
Sacrifice time -Fetuses	GD20	-	-
Sacrifice time -Pups	-	-	PND 4 PND 21

[a] Data obtained from pages 24-27 in the study report (MRID 49928301).
[b] All fetuses were weighed upon C-section removal on GD 20.
[c] Pups were pooled and weighed by sex on PND 0 and 4 (before and after culling).

3. **Sacrifice and sample collection:**

a. GD 20 dams (Subset I): Following euthanasia by carbon dioxide inhalation, maternal blood samples (approximately 2 mL/dam) were collected from the vena cava. Afterwards, uteri were immediately removed for fetal blood collection. Blood (as much blood as possible) was collected from the umbilical vein for each fetus and pooled by litter. Blood was collected into uniquely labelled tubes without anticoagulant for analysis of Triiodothyronine (T3), Thyroxine (T4), and Thyroid Stimulating Hormone (TSH).

Following fetal blood collection, each fetus was euthanized by a subcutaneous injection of sodium pentobarbital in the scapular region. The abdominal and thoracic cavities were exposed with a single incision (care was taken to avoid damaging the thyroid glands) and all fetuses, in each litter, were preserved *in situ* in 10% neutral-buffered formalin.

Each maternal female was then subjected to a gross necropsy. Maternal livers and thyroid glands were removed and weighed (thyroid glands weighed post-fixation). The thyroids and sections of the liver were preserved in 10% neutral-buffered formalin. Pregnancy status was determined for each female. Maternal carcasses were then discarded.

b. PND 4 pups: Blood samples (as much as possible) from culled pups (up to 20 litters/group in Subset II) were collected via cardiac puncture under isoflurane anesthesia and pooled by litter. Afterwards, each pup was euthanized by a subcutaneous injection of sodium pentobarbital in the scapular region. The abdominal and thoracic cavities were exposed with a single incision (care was taken to avoid damaging the thyroid glands). Pup livers were removed, weighed, and preserved in 10% neutral-buffered formalin. The remainder of the carcass was preserved *in situ* in 10% neutral-buffered formalin.

c. LD 21 dams/PND 21 pups (Subset II): Following euthanasia by carbon dioxide inhalation, blood samples (approximately 2 mL/dam) were collected from the vena cava of each LD 21 dam. Each maternal rat was subjected to a gross necropsy. Maternal livers and thyroid glands were removed, weighed (thyroids weighed post-fixation), and preserved in 10% neutral-buffered formalin. Maternal carcasses were then discarded.

Blood samples (approximately 1-1.5 mL/sex) were collected via cardiac puncture under isoflurane anesthesia from 2 PND 21 pups/sex/litter (up to 20 samples/sex/group). In the event that the amount of blood collected from a given litter (2 pups/sex/sample) was less than volume indicated, any remaining pups from the same litter, of the same sex, were bled to generate an adequate sample volume and appropriate information was added to the study records. Afterwards, all surviving pups (including pups not used for blood collection) were euthanized by exsanguination (pups used for blood collection) or by carbon dioxide inhalation (all remaining pups). For all pups, the abdominal and thoracic cavities were exposed with a single incision (care was taken to avoid damaging the thyroid glands). Pup livers were removed and weighed, and the whole liver was preserved in 10% neutral-buffered formalin. The remainder of the carcass was preserved *in situ* in 10% neutral-buffered formalin.

Females that failed to deliver were euthanized on post-mating day 25 by carbon dioxide inhalation and subjected to a gross necropsy. Uteri were opened and placed in 10% ammonium sulfide solution for the detection of early implantation loss. Carcasses were then discarded.

4. Thyroid hormone evaluations: All blood samples for analysis of T3, T4, and TSH levels were collected into uniquely labelled tubes without anticoagulant. Samples were allowed to clot at room temperature prior to centrifugation. Serum was isolated in a refrigerated centrifuge, flash frozen in liquid nitrogen, and stored frozen at approximately -70°C. The serum samples were shipped on dry ice via overnight courier to Ani Lytics, Inc., Gaithersburg, MD, for analysis. Hormone samples were analyzed using ELISA methods.

Samples were analyzed for Total T4 and T3 using solid phase coated tube radioimmunoassay reagents obtained from MP Biomedicals, OH.

Samples were analyzed for Rat TSH by double antibody radioimmunoassay using reagents

obtained from National Hormone and Pituitary Program, UCLA, CA.

Details of the assays are described in Appendix 5 of the study report (MRID 49928301). All three assays employed enzyme linked immunosorbent (ELISA) techniques. The study report states that a standard curve is generated and the amount of hormone in the samples was determined from this curve. However, no calibration standards or validation procedures were presented.

1. **Histopathology:** Microscopic examination was performed on the thyroid gland (with parathyroid glands, if present) from animals as described below. Remaining thyroid glands and livers were saved for possible future histopathology.

GD 20 Maternal	All
GD 20 Fetal	1 fetus/litter (20 fetuses/group)
PND 4 Pup	1 pup/litter (20 pups/group)
LD 21 Maternal	All
PD 21 Pups	1 pup/sex/litter (20 pups/sex/group)

D. DATA ANALYSIS:

1. **Statistical analyses:** Each mean was presented with the standard deviation (S.D.), standard error (S.E.), and the number of animals (N) used to calculate the mean. Data obtained from nongravid animals were excluded from statistical analyses following the mating period. Where applicable, the litter was used as the experimental unit.

   All statistical tests were performed using WTDMS™ unless otherwise noted. Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1% and 5%, comparing each test substance-treated group and the positive control substance-treated group to the vehicle control group.

   Maternal and offspring body weights and body weight changes, maternal food consumption data, gestation lengths, numbers of former implantation sites, and unaccounted-for sites, live litter size, number of pups born, hormone values, and organ weights were subjected to a parametric one-way ANOVA to determine intergroup differences (Snedecor and Cochran, 1980 as cited by the study report). If the ANOVA revealed significant ($p<0.05$) intergroup variance, two-sample t-tests (Sokal and Rohlf, 1981 as cited by the study report) were used to compare the test substance-treated group and the positive control substance-treated group to the vehicle control group. Mean litter proportions (percent per litter) of males at birth and pup viability during the postnatal period were subjected to the Kruskal-Wallis nonparametric ANOVA test to determine intergroup differences (Kruskal and Wallis, 1952 as cited by the study report). If the nonparametric ANOVA revealed significant ($p<0.05$) intergroup variance, Dunn's test (Dunn, 1964) was used to compare the test substance-treated group and the positive control substance-treated group to the vehicle control group.

   Given the unacceptability of this study, the Agency is not commenting on the statistical analysis performed in this study. However, the registrant should consult the agency regarding appropriate statistical analysis methods once acceptable data to address this data gap are available.

2. **Historical control data:** Historical control data of reproductive performance were included in the study report.

3. **Positive control data:** A positive control using 5-propyl-2-thiouracil (5-PTU) was concurrently run with the study.

II. RESULTS:

A. PARENTAL TOXICITY (GESTATION AND LACTATION PHASES):

1. **Mortality and clinical observations:** No mortality was observed in the parental animals treated with vehicle control, positive control, or test material in both gestation and lactation phases. Test substance-related clinical findings included red material around the nose at approximately 1 hour following dose administration as early as GD 9 and red material around the nose and yellow and brown material around the urogenital and anogenital areas at the daily examinations sporadically throughout the treatment period. Other findings were infrequent hair loss on various body surfaces occurring at similar frequencies in the vehicle control group. In the positive control group, red material around the nose was noted for females at approximately 1 hour following dose administration as early as GD 10. No other clinical findings were noted.

2. **Body weight:** Body weight and body weight gain data are summarized in Table 3. Mean body weights and body weight gains were unaffected by thiabendazole administration during gestation and lactation. Only a statistically significant lower mean body weight gain for females in the 10 mg/kg/day thiabendazole group during GD 18-20 was noted. Mean body weights were unaffected; therefore, this was considered incidental. Other differences from the vehicle control group were slight and not statistically significant. Mean body weights and body weight gains were unaffected by positive control substance administration during gestation. Differences from vehicle control group were slight and not statistically significant.

TABLE 3. Thiabendazole: Mean (±SD) maternal body weight and body weight gain (g) [a]			
	Dose in mg/kg bw/day (# of dams)		
Gestation			
	0 – Control (38)	1 – PTU (39)	10 – thiabendazole (40)
	Body Weight (g)		
GD 6	289±14.1	288±12.2	285±11.9
GD 9	297±15.1	295±13.6	292±14.1
GD 15	331±17.3	328±13.7	324±16.3
GD 18	372±21.7	369±16.3	369±19.9
GD 20	413±24.8	405±19.5	404±23.7
	Body Weight Gain (g)		
GD 6-9	9±5.4	8±4.7	7±5.7
GD 9-12	16±6.8	16±5.0	16±5.7
GD 15-18	41±11.8	41±7.8	45±8.2
GD 18-20	40±11.6	36±7.5	**36±6.8***
GD 6-20	124±16.3	118±15.7	120±14.6
Lactation			
	0 – Control (18)	1 – PTU (19)	10 – thiabendazole (20)
	Body Weight (g)		
LD 1	306±20.7	304±23.4	301±18.8
LD 7	332±19.4	331±15.2	324±17.4
LD 14	343±20.8	347±19.9	344±17.8
LD 17	344±19.5	350±18.6	346±15.4
LD 21	340±14.2	341±17.2	343±11.1
	Body Weight Gain(g)		
LD 4-7	10±9.5	10±9.7	9±7.9
LD 7-10	3±9.2	**10±8.3***	9±9.2
LD 1-21	34±15.7	37±14.4	42±12.5

[a] Data obtained from pages 45-52 in the study report (MRID 49928301).
* Significantly different from control group 1 at 0.05 using two-sample t-test
Nongravid weight(s) not included in calculation of mean, Mean differences calculated from individual differences

3. <u>Food consumption</u>: Food consumption data are summarized in Table 4. Mean feed consumption of test material treated groups was statistically significantly lower than the vehicle control group during GD 6-15, but not during GD 15-20. In the positive control group, it was lower during GD 18-20. However, in the absence of corresponding effects on body weight gain or body weight, these differences were not considered adverse.

Mean food consumption, evaluated as g/animal/day, was comparable to controls in the thiabendazole and positive control groups during lactation. Differences from vehicle control group were slight and not statistically significant.

TABLE 4. Thiabendazole: Mean (±SD) food consumption (g/rat/day) [a]			
	Dose in mg/kg bw/day (# of dams)		
Gestation			
	0 – Control (38)	1 – PTU (39)	10 – thiabendazole (40)
	±	±	±
GD 6-9	23±2.7	21±2.0**	21±2.4**
GD 9-12	24±2.6	22±1.9**	22±2.2*
GD 12-15	25±2.7	23±1.9**	23±2.1
GD 15-18	27±3.5	25±2.3*	26±2.2
GD 18-20	27±3.4	26±2.7	27±2.5
GD 6-20	25±2.4	23±1.7**	24±1.6*
Lactation			
	0 – Control (18)	1 – PTU (19)	10 – thiabendazole (20)
LD 1-4	35±5.9	36±4.9	36±4.5

TABLE 4. Thiabendazole: Mean (±SD) food consumption (g/rat/day) [a]			
LD 4-7	44±4.8	44±4.0	43±3.0
LD 7-10	51±5.2	54±4.8	52±4.3
LD 14-17	58±6.8	60±7.5	60±4.8
LD 17-21	66±9.5	65±10.9	67±6.4
LD 1-21	53±4.2	54±4.4	54±2.8

[a] Data obtained from pages 53-56 in the study report (MRID 49928301).
* Significantly different from control group 1 at 0.05 using two-sample t-test
** Significantly different from control group 1 at 0.05 using two-sample t-test

4. **Pregnancy and Prenatal Data:** Limited data was presented in the report on pregnancy and prenatal data. Gestation length among all groups was comparable. The mean gestation length in all groups was 21.7 days, compared to a mean gestation length of 21.8 days in the WIL Research historical control data. No signs of dystocia were noted in any group. The number of implantation sites was comparable among all group ranging from 15.2 to 15.6 per dam.

5. **Litter Data:** The mean number of pups born, live litter size, percentage of males per litter at birth, and postnatal survival between birth and PND 0 (relative to number born), PND 0-1, 1-4 (pre-selection), 4 (post-selection)-7, 7-14, 14-21, and from birth to PND 4 (pre-selection), and PND 4 (post-selection) to PND 21 were unaffected by the maternal thiabendazole or positive control substance administration (Table 5).

   The general physical condition (defined as the occurrence and severity of clinical findings) of all pups in this study was unaffected by maternal thiabendazole or positive control substance administration. Pups (litters) that were found dead were 6(5), 4(4), and 6(5) in the vehicle control, positive control substance, and 10 mg/kg/day thiabendazole groups, respectively. Four, 3, and 3 pups in the same respective groups were missing and presumed to have been cannibalized.

TABLE 5. Thiabendazole: Mean (±SD) pup data [a]			
	Dose in mg/kg bw/day (# of dams)		
	0 – Control (18)	**1 – PTU (19)**	**10 – thiabendazole (20)**
Litter Data Survival			
Litter number	18	19	20
Number born/Litter	14.4±2.52	14.2±2.50	15.0±2.06
% Males / Litter	49.9±7.69	52.4±12.82	53.6±11.20
Live Litter Size PND 0	14.1±2.42	13.9±2.44	14.8±2.20
PND 0 : (%/Litter)	98.2±3.59	98.6±2.85	98.6±3.70
PND 0-1	99.6±1.68	99.3±1.98	99.3±2.20
PND 1-4 pre culling	99.3±2.03	100.0±0.00	99.1±3.17
PND 4 post-culling	98.6±1.04	99.3±2.87	100.0±0.00
PND 7-14	100.0±0.00	100.0±0.00	100.0±0.00
PND 14-21	100.0±0.00	100.0±0.00	100.0±0.00
Birth to PND 4 pre-culling	97.1±3.89	97.9±3.17	97.0±5.17
PND 4(post-culling) to PND 21	98.6±4.04	99.3±2.87	100.0±0.00

[a] Data obtained from pages Tables S22 and S23, pages71-73 in the study report (MRID 49928301).
* Significantly different from control group 1 at 0.05 using two-sample t-test
** Significantly different from control group 1 at 0.01 using two-sample t-test

6. **Postmortem results:**

   a. **Hormone analysis:** Thyroid hormone concentration data are summarized in Table 6 for Gestation dams (sacrificed on GD 20) and Lactation dams sacrificed on LD 22.

TABLE 6 Thyroid hormone data of parent animals: mean±SD (CV)N [a]			
	0 – Control	**5- PTU**	**Thiabendazole**
	GD 20 females		
T3 (ng/dL)	68.28±12.510 (n=17)	77.76±17.424 (↑13.9%)(n=18)	73.09±20.463(↑13.9%)(n=18)
T4 (µg/dL)	2.50±0.555 (n=11)	2.67±0.652 (↑6.9%)(n=16)	2.54±0.432 (↑1.6%)(n=9)
TSH (ng/mL)	2.87±1.432 (n=20)	3.87±1.444* (↑34.8%)(n=20)	2.44±0.861(↓15.0%)(n=20)
	Fetus		
T3 (ng/dL)	NA	NA	NA
T4 (µg/dL)	NA	NA	NA
TSH (ng/mL)	1.86±0.500	2.88±1.014** (↑54.8%)(n=20)	1.75±0.371 (↓5.9%)(n=20)
	LD21 females		
T3 (ng/dL)	77.00±19.595 (n=17)	69.11±9.335(↓10.2%)(n=18)	72.86±13.28 (↓5.4%)(n=18)
T4 (µg/dL)	3.82±0.483 (n=18)	3.66±0.626 (↓4.2%)(n=20)	3.58±0.622 (↓6.3%)(n=20)
TSH (ng/mL)	2.74±1.070 (n=18)	2.88±1.888 (↑5.1%)(n=20)	2.87±1.690 (↑4.7%)(n=20)

[a] Data obtained from pages Tables S12 and S13, pages 59-61 in the study report (MRID 49928301).
* Significantly different from control group 1 at 0.05 using two-sample t-test
** Significantly different from control group 1 at 0.01 using two-sample t-test
NA Not available

b. <u>Organ weights</u>: In test material groups, the terminal body weight, absolute and relative weight of thyroid with parathyroid and liver of dams were comparable with the vehicle control group. A treatment-related statistically significant increase in absolute and relative weight of thyroid with parathyroid was observed in dams of the positive control group as compared with the vehicle control group (Table 7).

TABLE 7. Terminal body weight and absolute and relative (to body weight) organ weight in GD 20 and LD 22 dams: Group mean values [a]			
	0 – Control	**5- PTU**	**Thiabendazole**
Nº of dams	20	20	20
	Absolute Weights GD 20 dams (grams)		
Body Wt. TS	±	±	±
Liver	16.94±1.346	16.75±1.415 (↓1.1%)	16.6±1.272 (↓2.0%)
Thyroid glands	0.0136±0.00302	0.0122±0.00289 (↓10.3%)	0.0126±0.00073 (↓7.4%)
	Absolute Weights LD 22 Dams (grams)		
Nº of dams	18	19	20
Body Wt. TS	340±14.2	341±17.2	343±11.1
Liver	17.63±1.316	17.65±1.711	**18.77±1.610*(↑6.5%)**
Thyroid glands	0.0158±0.00304	**0.0178±0.00292* (↑12.7%)**	0.0164±0.00414
	Relative Organ Weight (%)		
Liver	5.189±0.3079	5.267±0.3713	**5.475±0.4916***
Thyroid glands	0.005±0.0010	0.005±0.0010	0.005±0.0010

[a] Data obtained from pages Tables S18 and S19, pages 66-68 in the study report (MRID 49928301).
Modified statistics used. Control group 1 was compared to group 2; control group 1 was compared to group 3.
* Significantly different from control group 1 at 0.05 using two-sample t-test
** Significantly different from control group 1 at 0.01 using two-sample t-test

c. <u>Gross Pathology</u>: There were no macroscopic lesions observed in test material treated dams or positive controls sacrificed in both gestation and lactation phases.

d. <u>Histopathology</u>: No treatment-related microscopic lesions were observed in any test material parental animals or positive control animals in both gestation and lactation phases.

B. <u>FETAL TOXICITY</u>: The only data reported was the thyroid hormone measurements (Table 6 above)

C. PUP TOXICITY:

1. **Clinical Signs and Mortality**: No treatment-related clinical signs were observed in pups of the test material groups. No treatment-related clinical signs were observed in pups of the vehicle control and positive control group.

   Pup mortality was comparable among all groups. Pups (litters) that were found dead were 6(5), 4(4), and 6(5) in the vehicle control, positive control substance, and 10 mg/kg/day thiabendazole groups, respectively. Four, 3, and 3 pups in the same respective groups were missing and presumed to have been cannibalized.

2. **Pup Body Weight**: Data is presented in Table 8. Mean pup body weights and body weight gains were unaffected by maternal thiabendazole administration or PTU from birth through weaning. Lower mean body weight gains for males and females during the PND 7-10 and lower mean body weight gain for females PND 17-21 were seen in the thiabendazole treatment. Higher mean body weight gain for males during PND 4-7, higher mean body weight gains for males and females during PND 10-14, and higher mean body weights for males on PND 14 and 17 were seen in the PTU pups. Because the differences were transient and did not affect mean body weights, these results were considered incidental.

Table 8. Mean ±SD body weight and body weight gain of pups (g) [a]

	Control	PTU	Thiabendazole	Control	5-PTU	Thiabendazole
No. of litters	**18[b]**	**19**	**20**	**17**	**19**	**20**
	Body weight (g)					
	Males			Females		
PND 1	7.1±0.7	7.2±0.47	7.1±0.55	6.8±0.73	6.8±0.53	6.6±0.66
PND 4	9.9±1.28	10.1±0.91	9.7±0.88	9.4±1.28	9.5±0.85	9.0±1.02
PND 7	16.6±2.07	17.3±1.31	16.7±1.44	16.0±2.26	16.3±1.17	15.4±1.86
PND 10	23.9±2.52	24.7±1.52	23.5±1.66	23.0±2.89	23.5±1.73	21.6±2.37
PND 14	33.6±2.97	35.5±1.91*	33.7±2.25	32.5±3.34	34.3±2.20	31.6±2.95
PND 17	39.1±3.04	41.2±2.51*	39.2±2.50	37.8±3.45	39.7±2.88	36.7±3.14
PND 21	49.9±4.53	51.1±3.62	49.5±4.19	49.3±4.76	49.8±3.48	46.5±4.90
	Body weight Gain (g)					
PND 1-4	2.8±0.81	2.9±0.54	2.6±0.56	2.6±0.81	2.7±0.62	2.4±0.59
PND 4-7	6.7±0.97	7.3±0.62*	7.0±0.79	6.5±1.2	6.9±0.65	6.4±0.93
PND 7-10	7.2±0.70	7.4±0.79	6.8±0.57*	7.1±0.71	7.2±0.89	6.3±0.75**
PND 10-14	9.8±1.34	10.8±1.00**	10.3±1.12	9.5±1.15	10.7±0.88**	10.0±0.98
PND 14-17	5.6±1.13	5.7±1.31	5.5±1.19	5.3±1.08	5.5±1.31	5.1±0.94
PND 17-21	10.8±2.30	9.9±2.39	10.3±2.46	11.5±2.05	10.1±2.20	9.9±2.66*

[a] Data obtained from pages Tables S25 and S26, pages 76-82 in the study report (MRID 49928301).
[b] Number of litters in the controls was 17 for PND 1.
Modified statistics used. Control group 1 was compared to group 2; control group 1 was compared to group 3.
* Significantly different from control group 1 at 0.05 using two-sample t-test
** Significantly different from control group 1 at 0.01 using two-sample t-test

3. **Postmortem results:**

   a. **Hormone Analysis**: Pup serum thyroid hormone data is presented in Table 9. Mean T3, T4, and TSH levels for F1 pups on PND 4 and PND 21 were unaffected by maternal thiabendazole administration (T4 levels for all F1 pups on PND 4 were below the level of quantification). On PND 21, the group mean TSH value for male pups in

this group was slightly lower than the vehicle control group. Although, this difference from the vehicle control group achieved statistical significance, it is considered incidental and not treatment-related. A treatment related effect on thyroid function would follow an expected pattern of feedback inhibition, *i.e.* when T3 and T4 concentrations are low, TSH levels are expected to be elevated and, conversely, when T3 and T4 concentrations are high, TSH release from the pituitary is downregulated. However, there is no corresponding increase in T3 or T4 in F1 male pups on PND 21 following thiabendazole administration.

The positive control substance did not elicit the expected responses in T3, T4, or thyroid stimulating hormone (TSH) levels. On PND 4, the group mean TSH value was statistically significantly higher than the vehicle control group. However, this was considered incidental to treatment because the increase does not follow the expected patterns of negative feedback inhibition, *i.e.*, TSH levels are expected to be elevated when T3 and T4 concentrations are low; However, there is no corresponding decrease in T3 in F1 pups on PND 4 following PTU administration.

TABLE 9 Thyroid hormone data of pups: mean±SD (% change)No. [a]			
	0 – Control	**1 – PTU**	**10 – thiabendazole**
	PND 4 PUPS		
T3 (ng/dL)	50.43±0.220 (2)	61.23±9.710 (↑21.4%) (6)	52.85±3.444 (↑21.4%) (3)
T4 (µg/dL)	NA	NA	NA
TSH (ng/mL)	0.82±0.151 (13)	**1.30±0.427** (↑58.5%) (18)**	0.94±0.283 (↑18.3%) (19)
	PND 21 PUPS MALES		
T3 (ng/dL)	109.81±19.168 (18	120.25±23.006 (↑9.5%) (19)	108.69±13.343 (↓1.0%) (20)
T4 (µg/dL)	4.56±0.886 (18)	4.33±1.224 (↓5.0%) (19)	4.63±0.856 (↑1.5%) (20)
TSH (ng/mL)	1.38±0.338 (17)	1.33±0.309 (↓3.6%) (19)	**1.06±0.377* (↓23.2%) (20)**
PND 21 PUPS FEMALES			
T3 (ng/dL)	104.25±15.431 (18)	111.67±17.578 (↑7.1%) (19)	114.37±17.491 (↑9.7%) (20)
T4 (µg/dL)	4.21±1.165 (18)	4.16±1.099 (↓1.2%) (19)	4.76±1.268 (↑13.1%) (20)
TSH (ng/mL)	1.19±0.357 (18)	1.24±0.336 (↑4.2%) (19)	1.11±0.413 (↓6.7%) (20)

[a] Data obtained from pages Tables S27 and S28, pages 83-85 in the study report (MRID 49928301).
Modified statistics used. Control group 1 was compared to group 2; control group 1 was compared to group 3
* Significantly different from control group 1 at 0.05 using two-sample t-test
** Significantly different from control group 1 at 0.01 using two-sample t-test
Note: the lowest calibration standard (T3: 50 ng/dl, T4: 2 ug/dl, TSH: 0.39 ng/ml) was considered the LLOQ; values below the LLOQ were excluded from calculations

b. Organ weights:

Organ weight data for pups are summarized in Table 10. Terminal body weights and absolute and relative liver weights were comparable among all groups. Only liver weights were reported, thyroid/parathyroid weights were not reported.

TABLE 10. Pups: Terminal body weight and absolute and relative (to body weight) organ weights: Group mean values (mean of means) [a]						
	Control	5-PTU	Thiabendazole	Control	5-PTU	Thiabendazole
	Males PND 4			**Females PND 4**		
Number	17	17	20	17	16	17
BW (G)	9.7±1.21	10.1±1.05	9.7±0.98	9.2±1.00	9.6±0.96	9.0±1.06
Liver	0.354±0.0381	0.366±0.0424	0.347±0.0402	0.346±0.0340	0.354±0.0387	0.341±0.0460
Liver Relative Wt	3.680±0.3050	3.626±0.2854	3.619±0.4308	3.810±0.2965	3.730±0.4168	3.797±0.4182
	Males PND 21			**Females PND 21**		
Number	18	19	20	18	19	20
BW (G)	49.9±4.53	51.1±3.63	49.5±4.19	49.3±4.76	49.8±3.48	46.5±4.90
Liver	2.41±0.287	2.37±0.295	2.42±0.344	2.43±0.280	2.35±0.262	2.28±0.321
Liver Relative Wt	4.809±0.2253	4.624±0.3771	4.874±0.3744	4.926±0.2512	**4.718±0.3352***	4.893±0.3612

[a] Data obtained from pages Tables S29 and S30, pages 86-89 in the study report (MRID 49928301).
Modified statistics used. Control group 1 was compared to group 2; control group 1 was compared to group 3
* Significantly different from control group 1 at 0.05 using two-sample t-test

c. Gross Pathology: Macrosopic examinations of pups were not done or reported.

d. Histopathology: There were no test substance- or positive control substance-related changes in the thyroid or parathyroid (when present for evaluation) glands in any of the animals (male and female pups at PND 4, and male and female pups at PND 21). Microscopic changes observed in the thyroid glands (ultimobranchial cyst and ectopic thymus) in male and female pups at PND 21 were considered spontaneous findings that were either present in controls with similar distribution or showed minimal higher incidence in test substance- or positive control substance-treated groups that was consistent with normal biological variation.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: Thyroid function was unaffected by maternal thiabendazole administration. Following exposure to thiabendazole at 10 mg/kg/day during gestation or lactation, there were no test substance-related alterations in T3, T4, or thyroid stimulating hormone (TSH) levels, thyroid gland or liver weights, or microscopic changes in the thyroid and parathyroid glands of F0 females on GD 20 (Subset I) or LD 21 (Subset II), F1 fetuses on GD 20 (Subset I), or F1 pups on PND 4 or PND 21. The lower group mean TSH level in PND 21 male pups, which achieved statistical significance relative to controls, was considered incidental and not treatment-related. A treatment related effect on thyroid function would follow an expected pattern of negative feedback inhibition *i.e.* when T3 and T4 concentrations are low, TSH levels are expected to be elevated, and, conversely, when T3 and T4 concentrations are high,

TSH release from the pituitary is downregulated. However, this did not happen, correlating with the lack of any pathological changes in the thyroids of these animals. Clinical effects, body weight effects or food consumption effects were minimal and not adverse.

The positive control substance (PTU) did not elicit the expected responses in T3, T4, or thyroid stimulating hormone (TSH) levels, thyroid gland or liver weights, or microscopic changes in the thyroid and parathyroid glands of F0 females or F1 fetuses/pups. The investigators attributed the lack of the positive control response to a possible lower dose of PTU.

B. REVIEWER COMMENTS: This study was conducted using one dose of thiabendazole (10 mg/kg/day) along with a vehicle control and a positive control group based on discussions with EPA (TXR 0057235). EPA required that the positive control agent should produce at least a 30% response but not greater than 50%. The study used 5-propyl-2-thiouracil (PTU) at a dose level of 1.2 mg/kg/day in corn oil, based on a published study by Axelstad *et al.* 2008 (Developmental Neurotoxicity of Propylthiouracil (PTU) in Rats: Relationship Between Transient Hypothyroxinemia During Development and Long-Lasting Behavioural and Functional Changes. *Toxicology and Applied Pharmacology* **2008**, *232*, 1-13). The Axelstad study employing a comparable dosing regimen, demonstrated a decrease in T4 levels of approximately 50% in PND 16 pups, relative to controls. At this PTU dose level, a 47% increase in thyroid weight, along with histopathological changes (15/33 animals) were noted

in the PND 16 pups. This dosing regimen was used in the current study as the dose route and frequency mimics that of the test substance. However, the EPA reviewer notes that the Axelstad *et al.* 2008 paper used 6-Propyl-2-thiouracil (CAS 51-52-5). 6-Propyl-2-thiouracil is a potent antithyroid agent, is used in the treatment of hyperthyroidism, and is the standard positive control agent used in developmental thyroid studies.

There were no adverse effects on body weight, body weight gain, or food consumption in adult female treated with thiabendazole by oral gavage at 10 mg/kg/day during the gestation and lactation phase. No mortality or adverse clinical toxicity was observed either in the thiabendazole treated animals or the PTU positive control animals.

There were no effects of thiabendazole, or the positive control substance on mean maternal gestation length, the process of parturition or the growth, survival, and clinical condition of F1 pups during the postnatal period. In addition, there were no noted treatment-related macroscopic findings in adult animals.

Thiabendazole at 10 mg/kg/day at PTU at 1.2 mg/kg/day did not impact the serum thyroid hormone levels. The serum thyroid hormone levels of dams (gestation and lactation) in the test material treated groups and positive control were comparable with the concurrent vehicle control group. There were no test substance-related alterations in T3, T4, or thyroid stimulating hormone (TSH) levels, thyroid gland or liver weights, or microscopic changes in the thyroid and parathyroid glands of maternal animals (GD20/LD21), fetuses, or pups on PND 4 /PND 21. In the absence of a response from a positive control agent, the agency cannot determine the accuracy, reliability, or sensitivity of the assay used. The testing facility has failed to demonstrate the ability to detect changes in thyroid hormones at any lifestage. Consequently, the study is considered unacceptable consistent with the guidance and protocol review provided by the agency to the registrant prior to the conduct of this study.

This developmental thyroid toxicity study in the rat (pregnant, fetus, offspring) is classified **Unacceptable/Non-Guideline**. The positive control agent 5-PTU used in the study did not produce the expected effects on the thyroid/parathyroid and the T3, T4, TSH hormones. Therefore, in the absence of positive findings by the positive control agent, the results of the test material are not reliable.

The registrant submitted a separate paper titled “Thiabendazole – Validity of the Developmental Thyroid Toxicity Study Assessment” (MRID 49928302) explaining the lack of the positive control effects in the study and the validity of the test compound results. The paper states that “the expected response to PTU treatment was not observed. Either the dose was not adequate for the rat’s genetics/stock/strain, or the rats did not receive the proper dose due an undetected problem with the PTU chemical and/or formulation”. The paper adds “Unfortunately, WIL laboratories has previously administered PTU at a low dose level of 3.8 mg/kg/day; this dose level is approximately 3-fold greater than that used in the present study. At 3.8 mg PTU/kg/day, the following changes were noted (expressed as a percentage change from control)”.

	PND 4 (♂+♀ combined)	PND 28 (males)	PND 28 (females)
T3	-25%	-24%	-12%
T4	-96%	-33%	-28%
TS	+478%	-13%	+40%

The EPA reviewer notes that the current thiabendazole study employed 5-Propyl-2-thiouracil (CAS Number 2954-52-1). The WIL Laboratories data presented in the above table does not specify if the 5-PTU or the 6-PTU were used. The study report based the use of this chemical on a published paper by Axelstad *et* al, 2008. The Axelstad study employing a comparable dosing regimen, demonstrated a decrease in T4 levels of approximately 50% in PND 16 pups, relative to controls. At this PTU dose level, a 47% increase in thyroid weight, along with histopathological changes (15/33 animals) were noted in the PND 16 pups. This dosing regimen was used in the current study as the dose route and frequency mimics that of the test substance. However, the EPA reviewer notes that the Axelstad *et al.* 2008 paper used 6-Propyl-2-thiouracil (CAS 51-52-5). Therefore, the additional explanation by the registrant (MRID 49928302) is not satisfactory and the thiabendazole developmental thyroid study (MRID 49928301) remains **Unacceptable/Non-Guideline** and failed to demonstrate the objective of the study. The objective of the study was to detect the potential of thiabendazole to adversely impact thyroid function of adult female rats during gestation and lactation, and their offspring prenatally and postnatally, consequent to exposure of the adult female from implantation (GD 6) through weaning of the offspring (PND 21). The study did not achieve its stated objective.

C. STUDY DEFICIENCIES: The following are major deficiencies that make the study unacceptable:

- The positive control agent used did not produce the intended effect.
- Pups were not dosed directly. It was assumed pups were exposed in utero and postnatally. No data was presented to demonstrate this.
- Limited fetal data was presented. Only hormonal data was presented.